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Multigeneration Maternal Transmission in Italian Families With Neural Tube Defects

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Periconceptional vitamin supplementation with folate prevents about three-quarters of expected cases of neural tube defects (NTDs) in clinical trials. However, vitamin action may be regulated at the level of the gene, and individual susceptibility to environmental agents, including dietary components, also may be under genetic control. We investigated the presence of familial factors in a retrospective case control study of neural tube defects in Genoa, Italy. Cases included all patients treated at a single pediatric neurosurgical service. Controls matched on age and sex came from the same hospital. We found strong evidence for the contribution of genetic factors in this study. There was an excess risk of 14 for the occurrence of NTDs in first-degree relatives compared to controls ($P < .0005$). There was no difference in sex ratio in any group of relatives, but maternal grandparents of children with a high spinal lesion had 14% fewer offspring than paternal grandparents ($P < .005$), possibly because of excess miscarriages. Our study is the first to show complex patterns of inheritance in spina bifida families affecting three generations in one clinical subgroup and preferentially on the mother's side. These results support a role for genomic imprinting and highlight the value of multidisciplinary epidemiologic and clinical studies that include multiple generations. New studies incorporating dietary and genetic approaches will help clarify and extend these findings. © 1996 Wiley-Liss, Inc.

KEY WORDS: family studies, neural tube defects, spina bifida, birth defects, epidemiology, family size

INTRODUCTION

Neural tube defects (NTDs) are one of the most common congenital malformations in newborn children, occurring in 5 of every 10,000 births in Italy during the period 1980–1988. Rates throughout Europe are generally similar to those in Italy, with the notable exception of areas in Western Europe including the Republic of Ireland. In Dublin, the rate of NTDs in live births was 22.2 per 10,000 over the same period, 1980–1988 [Eurocat, 1991a].

In many European regions, a downward trend in the occurrence of neural tube defects has been noted [Eurocat, 1991b]. In part, this reflects increasing use of prenatal diagnosis, but where prenatal diagnosis is not available, as in Ireland, the steep fall in NTD rates cannot be attributed to medical intervention, but may reflect dietary improvements.

The causes of NTDs are undoubtedly complex. NTDs may be the end result of a number of different processes, but the prevailing view is that nonsyndromic cases are multifactorial, perhaps polygenic, with an environmental component [Neumann et al., 1994]. The last 10 years have brought a breakthrough in our understanding of the causes and prevention of NTDs. Following extensive evidence for the role of dietary supplementation with folic acid before and during pregnancy in preventing the occurrence of NTDs, clinical trials showed that administration of folic acid prevents about three-quarters of NTDs [MRC, 1991; Czeizel and Dudas, 1992].

However, it is unlikely that folic acid deficiency by itself causes all NTDs. The best evidence comes from the fact that clinical trials failed to prevent one-quarter of occurrences. Other events such as drug exposures and hyperthermia [Rosa, 1991; Lynberg et al., 1994] may also lead to NTDs. Although intriguing clues exist in the older literature, the role played by genetic susceptibility in the origins of NTDs is still to be clarified.

The genetic influence in NTDs is evident in the predominance of affected females, the increased risk to other sibs, which is higher in high rate areas than in low rate areas [Elwood et al., 1992], the declining risk in other relatives with increasing genetic distance [McManus, 1987], and the altered sex ratio in transmitting relatives [Mariman and Hamel, 1992]. Further,

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NTDs occur as components of euploid and aneuploid syndromes, among them Meckel syndrome [Holmes et al., 1976; Keohane et al., 1988], Waardenburg syndrome [Byrne and Warburton, 1986; Tassabehji et al., 1992].

The influence of dietary and other environmental factors may be controlled at the level of DNA, and it may be that molecular studies would be most fruitful in explaining alterations in mechanisms that lead to a NTD. The tools of modern molecular studies that have proved so useful in understanding other conditions may help clarify the developmental origins of these complex deformities.

To evaluate the extent to which the genetic background contributes to the occurrence of NTDs in a low rate area such as Italy, we designed a case control family study in Genoa. Both cases and controls were identified from families seeking treatment at the G. Gaslini Children's Hospital in Genoa. With 800 beds, the hospital is the largest children's hospital in Italy.

This report examines differences between cases and controls in a set of factors considered to indicate the presence of a genetic condition. These are risk of a neural tube defect in other family members, altered sex ratio, and differences in sibship size.

MATERIALS AND METHODS

Cases With Neural Tube Defects

Cases consisted of all patients who ever sought treatment for any neural tube defect at the neurosurgical clinic since its inception in 1976. Two groups of patients were pooled for this study. The first group was ascertained retrospectively and consisted of all patients coming to the G. Gaslini Hospital for any treatment for any neural tube defect from January 1, 1976 until September 30, 1991 (N = 354). The second series was identified prospectively between October 1, 1991 and December 31, 1992 (N = 98). A total of 452 patients was identified.

We attempted to follow up each of the 452 patients for interview and to obtain blood samples. Patients in active follow-up were scheduled for an appointment and the families interviewed by one of the study personnel. Some patients were known to be dead; their families were traced and contacted to obtain the date and cause of death only. No interview information was obtained from the families of deceased patients.

Study personnel implemented tracing procedures for patients who had not been seen in some time. In order to trace patients no longer in active follow-up, we obtained their last known address from the files. For most patients, a simple phone call sufficed to locate them. For others, it was necessary to contact the local government officials in their community of last residence to obtain the new address or the date of death of deceased patients. Former patients were either telephoned or were sent a letter inviting them to participate in the study.

The results of the contact procedures were as follows: 8 patients were found to be ineligible, because 3 were adopted and 5 had been abandoned by their parents; 40 patients were dead; 14 had no address, or the patient came from a foreign country. Of 119 patients no longer in active follow-up, 17 refused by letter, 52 refused by phone, and 50 agreed to come into the clinic for medical examination and interview. Of 271 patients in active follow-up, all except 8 agreed to participate in the study. Thus we had a response rate from cases of 80.3% (313/390). The 313 cases in this study comprised 150 males and 163 females. The distribution of diagnoses of interviewed cases did not differ from that of cases who were not interviewed.

Interviews

All case interviews were done in the neurosurgical clinic by study personnel between March 3, 1992 and December 31, 1993, except for three interviews via telephone. In ~80% of interviews, both parents were present and responded on behalf of their child. The oldest patient was a 36-year old woman, who was interviewed personally.

The interview covered basic demographic characteristics of the parents, activities of daily living appropriate for age, and work experience for cases over age 15. We asked about the mother's pregnancy history, exposures before and during each pregnancy, and the health of each child, especially the presence of birth defects. Family history questions related to the case's parents and grandparents, the brothers and sisters of each parent, and first cousins. Total number of first cousins was not asked. Each questionnaire carried a blank pedigree diagram used to complete the family information.

We accepted both case and control parents' reports regarding the existence of other malformations in their children or in other family members without seeking confirmation. However, information on the case child's NTD diagnosis was obtained from the clinical record.

Blood Samples

Blood from each case (3–4 ml) and each parent (5–6 ml) was collected. The lymphocytes were isolated with Ficoll separating solution and washed with normal saline. The cells were suspended in fetal calf serum and 10% DMSO and stored in liquid nitrogen. Samples from 749 individuals representing 286 families were stored. Cytogenetic studies were done on tissue obtained from the NTD lesion at the time of surgery and the results are reported separately [Haupt et al., 1995].

Control Group

Controls were identified from patients coming to the G. Gaslini Children's Hospital (IGG) for tonsillectomy (N = 133), orthopedic problem (N = 31), gastrointestinal upset (N = 26), odontology (N = 17), urinary tract infection (N = 15), upper respiratory tract infection (N = 15) and fever (N = 13), and 23 infants from the neonatology service. Other diagnoses included cranial trauma, emergency surgery, dermatology, growth delay without growth hormone deficiency, anemia, and cardiovascular infection. All but 10 controls were identified at IGG. Because of difficulties matching older children, 10 controls came from another hospital.

Controls were matched on age within 4 months, and on sex, and on region of residence when possible, in that order. Those families who refused our request for an in-

TABLE I. Neurosurgical Diagnoses and Level of Lesion of Interviewed Cases

	Interviewed cases (N = 313)
1. Cranial neural tube defects	25 (8.0%)
—Meningocele	16
—Dermal sinus	6
—Cephalocele	3
2. Spinal neural tube defects	
(a) Open spinal dysraphism	157 (50%)
—Myelomeningocele with progressive hydrocephalus	113
—Myelomeningocele with nonprogressive hydrocephalus	28
—Myelomeningocele without hydrocephalus	13
—Myelomeningocele with multicavitary hydrocephalus	3
(b) Occult spinal dysraphism	131 (41.9%)
—Dermal sinus	34
—Lipoma of the spinal cord	26
—Caudal dysgenesis	20
—Chiari I	12
—Asymptomatic spina bifida	11
—Lipomyelomeningocele	11
—Meningocele	7
—Dysontogenetic mass	6
—Tethered cord	3
—Hydromyelia	1
Total	313
Level of lesion in 212 relevant cases	
High	23 (10.9%)
Medium	137 (64.6%)
Low	52 (24.5%)

terview were replaced by another family to obtain a one-to-one match. In all, 313 controls were enrolled. All were matched on age and sex. Due to referral patterns, matching on region was not achieved in all cases.

The controls were asked the same questions about their family history of birth defects as the cases. No clinical records were obtained. They were not asked about their schooling, activities of daily living, and health, since these questions were directed toward the problems of children with neural tube defects. The controls were interviewed by the same interview team as the cases, between March 20, 1993 and October 11, 1993.

Statistical Methods

Differences between groups of interest were compared for statistical significance using the Chi-square test and Fisher's exact test when appropriate. Analysis of variance techniques were used to adjust for potential confounding effects.

RESULTS AND DISCUSSION

Description of Population

When the principal neurosurgical diagnoses of 313 interviewed cases were classified according to the scheme suggested by Naidich and colleagues [McLone and Naidich, 1989; Naidich et al., 1992], 25 (8%) had cranial defects (meningocele, dermal sinus, and cephalocele), a further 157 (50%) had open neural tube defects, or myelomeningocele, and the balance (131, 41.9%) had a variety of closed defects of the spine, including dermal sinus, lipoma, and caudal dysgenesis (Table I).

Cases showed the expected excess of females, 52.1% of the total (Table II), even though this is a referral series. There were no differences between cases and controls in their average age at interview and the average ages of the mothers and fathers at the time of interview (Table II). However, differences between the two groups in their region of residence were considerable. For instance, almost three-quarters of the controls, but only

TABLE II. Characteristics of Cases and Controls

	Cases	Controls
Sex, males:females	150/163	150/163
% female	52.1%	52.1%
Age of case in years		
mean (SD)	7.4 (5.9)	7.3 (5.9)
median, range	6.0, 0–36	5.7, 0–36
Age of mother in years		
mean (SD)	35.4 (8.1)	35.3 (7.1)
Age of father in years		
mean (SD)	39.4 (8.2)	39.0 (7.9)
Region of family's residence		
North (Liguria, Piemonte, Emilia Romagna, Lombardy)	115 (36.7%)	255 (81.5%)
Center (Tuscany, Lazio, Abruzzo, Umbria, Marche)	31 (9.9%)	4 (1.3%)
South (Sicilia, Puglia, Calabria, Sardegna, Campania, Basilicata, Molise)	167 (53.4%)	54 (17.3%)
Total	313 (100%)	313 (100%)

TABLE III. Sex Ratio of NTD Families and Control Families*

	NTD cases Males, females	Controls Males, females	*P*
Brothers and sisters of subject	181, 160	140, 120	NS
% male	53.1%	53.8%	
Fathers' brothers and sisters	505, 532	362, 355	NS
% male	48.7%	50.5%	
Mothers' brothers and sisters	445, 453	372, 360	NS
% male	49.6%	50.8%	

*NS = not significant.

one-quarter of the cases, came from Liguria, the region of Italy where Genoa is located. In contrast, over half (53%) of the cases came from the south of Italy, compared to only 17% of controls, reflecting referral patterns. Many of the specialty clinics at the G. Gaslini Children's Hospital draw from a wide referral base, especially from the south of Italy. The control series comes mostly from Liguria, indicating the relatively benign nature of the conditions for which they sought medical attention. These regional differences are important and we attempted to account for them in the analyses.

Sex Ratio

There were slightly more males than females among sibs of cases and controls (Table III) slightly fewer males than females among sibs of case parents, and slightly more males than females among sibs of control parents. However, none of these differences achieved statistical significance. An unexpected finding in this analysis of sex ratio was the reduction in the expected number of sibs of case mothers compared to sibs of case fathers. The number of sibs of case mothers was statistically significantly less than the number of sibs of case fathers ($P < .001$). This finding is analysed further below. The size of control families is smaller than case families reflecting different fertility patterns in the industrial urbanized north of Italy and the more rural south.

Other Neural Tube Defects in Families of Affected Children

Among the 341 brothers and sisters of the 313 cases, there were 6 sibs (1.8%), all from different families who also had a neural tube defect (Table IV). This was a significant excess compared to controls ($P < .04$), none of whom had a NTD. Among 616 case parents, 4 fathers and 5 mothers had occult spina dysraphism, including one family where both the father and mother of the child were affected.

Among all first-degree relatives (fathers, mothers, and siblings) of cases, 15.5 per 1,000 had a NTD, compared to 1.1/1,000 among controls ($P < .0005$), a 14-fold excess risk. Second-degree relatives of cases still had a threefold excess risk of a NTD, not a statistically significant difference.

Relationship Between Genetic Factors

At the conclusion of the a priori hypothesis testing, a number of genetic factors seemed linked to the occurrence of neural tube defects in this group of families. These were smaller sibship size in case mothers than case fathers, and a strong recurrence risk in first-degree relatives. To see if some of these factors were related to either clinical variables or other genetic variables, a stratified analysis was performed (Table V). Since the numbers of families with recurrences was small, the variable compared in these strata was par-

TABLE IV. Spina Bifida in Relatives of NTD Cases and Controls*

	Cases	Controls
First-degree relatives (sibs and parents)		
Fathers	4/313	1/313
Mothers	5/313	0/313
Siblings	6/341	0/260
All first-degree relatives	15/967	1/886
Rate	15.5/1,000	1.1/1,000 $P = .0005$
Relative risk	14	
Second-degree relatives (grandparents, uncles, aunts)		
Grandparents	1/1,252	0/1,252
Paternal uncles and aunts	1/1,034	1/717
Maternal uncles and aunts	2/897	0/732
All second-degree relatives	4/3,183	1/2,701
Rate	1.3/1,000	0.4/1,000 $P = .2$
Relative risk	3	

*Persons with unknown values not included.

TABLE V. Average Sibship Size on Fathers' and Mothers' Side by Potential Genetic Risk Factors*

	Fathers	P	Mothers	P
Total sibship size	4.3		3.9	<.001
Sex of affected child				
Male	4.3		3.8	
Female		4.3 NS	3.9	NS
Recurrence in first-degree relative				
Yes (N = 13)	4.4		3.7	
No (N = 300)		3.8 NS	4.3	NS
Site of lesion				
Cranial (N = 25)	4.3		4.0	
Spinal (N = 288)		4.3 NS	3.9	NS
Level of spinal lesion				
Thoracic (D12)				
Yes (N = 23)	3.9		2.8	
No (N = 191)		4.5 NS	4.1	<.005

*NS = not significant.

ents' sibship size. The hypothesis here is that factors indicating the presence of a genetic disease should occur together in the same families. For instance, we would expect that in families with other affected children, mothers' sibship size would be smaller than in families with no other affected children. Sibship size in mothers and fathers was examined within levels of a number of variables—sex of the affected child, whether the recurrence was in the mother, father, sibling, or all together, location and type of NTD, presence of malformations, whether the NTD was isolated or part of a complex of malformations. No factor was associated with smaller sibship size in fathers, and only one was associated in mothers. Among the 214 families with a spinal lesion where the involved vertebral level could be identified using functional criteria, the 23 families with thoracic functional level (or high defects) had significantly smaller mothers's sibships than in families with NTDs at other locations ($P < .005$). Sibship size in families with lower lesions was closely similar in mothers' and fathers' families (4.1 vs 4.5, NS).

Both case and control fathers were 4 years older than mothers (Table II). An analysis of variance that included fathers' age showed strong secular trends for fertility declines in Italy in both cases and controls, but fathers' age did not explain the sibship size difference between case fathers and mothers.

Since we asked for year of birth of parents' brothers and sisters, we could evaluate the length of the interval between each birth, a possible indicator of recognised or unrecognised miscarriages. The interbirth interval was calculated from the youngest to the oldest child. Only liveborn children were included. On the mothers' side 1,211 sibs, including the mother, gave 898 intervals. On the fathers' side, 1,350 sibs, including the father, gave 1,037 intervals. There was no difference overall in the interbirth interval between mothers and fathers (3.4 vs. 3.37 yr). When the data were divided by level of lesion for mothers' families only, families with a high lesion (N = 23) had an interbirth interval of 3.98 years compared to 3.4 years for lower lesions ($P < .08$). One interpretation of this difference of borderline statistical significance is that maternal grandparents of a child with a high NTD had more miscarriages than maternal grandparents of a child with a lower lesion.

The defects in these 23 cases consisted of meningomyelocele in 20 and caudal regression syndrome in three. When we evaluated sibship size in all 20 cases of caudal dysgenesis vs. all other cases, there was no evidence for a fertility deficit on the maternal side. There were no NTD recurrences in first-degree relatives of the 23 cases with a high defect.

Clearly, in these families, the mother of the child with a high spinal lesion carries some factor that links the reduced fertility of her parents with the presence of a NTD in her child. The presence of this factor could be indicated by an excess of miscarriages, or a paucity of liveborn children, or a longer interpregnancy interval. However, this analysis is complex and must be deferred for another study.

We have shown that in a region of Italy where NTDs occur at a low rate, evidence for the genetic origins of the disease is still strong. Families of a NTD child participating in this study were 14 times more likely than control families to have another affected member among their first-degree relatives. In addition, we found that mothers of the NTD child had fewer siblings than expected. This smaller family size in the maternal grandparents only occurred in families where the NTD was located in the thoracic functional region of the spine and was associated with a longer interbirth interval, suggesting an excess of miscarriages. There was no suggestion in this study of smaller family size in paternal grandparents. This study showed no statistically significant disturbances of the sex ratio in relatives of the NTD child.

Raised risk of NTD in relatives of the proband is a consistent finding of the epidemiology of NTDs [Elwood et al., 1992]. The rate in sibs in our study is 1.8% (6/341), close to the recurrence risk rate of 2.69% and to the 95% confidence interval of 1.93–3.45 given by Elwood et al. [1992, p. 611] for countries in Continental Europe in studies carried out between 1945 and 1982. The rate of NTDs among our controls is 5.8 per 10,000 (counting only sibs, aunts, and uncles), very similar to the rate for Emilia Romagna (the neighboring region to Liguria) of 5.3 per 10,000 livebirths [Eurocat, 1991a]. Recurrence risk is greater among mothers' relatives than among fathers' and has been linked to high spinal defects in other studies [Hall et al. 1988].

One of the strengths of this study is the inclusion of a control group. Although controls were not representative of the general population, the proportion of controls' first-degree relatives with a NTD is similar to expected rates [Eurocat, 1991a]. Rates of NTDs in case relatives may be inflated due to ascertainment and recall bias. Respondents' knowledge of the health status of their relatives and their spouses' relatives will be incomplete and inaccurate to some degree. However, studies of the reliability of family histories show them to be reasonably accurate for first-degree relatives and less reliable for second-degree relatives [Bondy et al., 1994].

In our study, maternal grandparents had 14% fewer children than expected, if we accept that the fathers'

families are the expected size. Since the controls were incompletely matched on region, we cannot be sure that the fathers' sibships are not also smaller than expected. Inclusion of another control group such as fathers' wives' brothers could address this issue. However, the fact that we found no characteristic associated with smaller family size in paternal grandparents suggests that using the fathers as a comparison group for mothers is appropriate.

Smaller family size is seen only in families of cases with a high spinal lesion, indicating the importance of obtaining accurate clinical information for epidemiological studies. In our study cases with a lesion located at the thoracic-lumbar functional level, whether meningomyelocele or caudal regression syndrome, were the only group linked to a fertility deficit, possibly due to excess miscarriages in the maternal grandparents. As far as can be determined from reviewing the literature, this is the first report indicating that a trait associated with one type of NTD can be traced back to a particular group of grandparents.

Our finding accords both with other studies and also with quite recent evidence for genomic imprinting. Mothers seem more likely to pass on the genetic susceptibility to their children than fathers. A common finding in many studies is the excess of affected relatives in mothers' families compared to fathers' families [Carter and Evans, 1973; Nevin and Johnston, 1980; Williamson et al., 1985; McManus, 1987]. In addition, McManus' study clearly shows a significantly greater proportion of affected relatives among both mothers' sisters and among mothers' sisters' children. Figure 1 shows the proportion of various types of relatives with NTDs in several large studies occurring in different times and places. Each group is ordered according to the closeness of relationship to the affected child, from left to right. Not all groups of relatives were listed in each study. If Mendelian inheritance applied, one would expect the risk to radiate out from the affected person, declining as it goes, so that first-degree relatives are more likely to be affected, followed by second-degree relatives, and rates in third-degree relatives would be lowest. Instead, each study shows that the risk actually *increases* as the degree of relationship decreases, culminating in the highest rate among third-degree relatives, mothers' sisters' children. The consistency of these findings is remarkable and has puzzled investigators. But in the light of multigeneration maternal inheritance, this pattern becomes readily interpretable.

We propose that the maternal grandparents pass on to their daughters a trait (indicated by a circle in Fig. 2) that results in a NTD in grandchildren. The grandparents either fail to pass it on to their sons, or the sons fail to transmit it to their children, perhaps because the trait requires an intrauterine environment for expression, or because the sons themselves are infertile (Byrne and Reilly, submitted for publication). Thus in families ascertained because of a NTD child, some studies show that the first cousins of that child related via their mothers are more likely than other types of first cousins or other relatives to have a NTD.

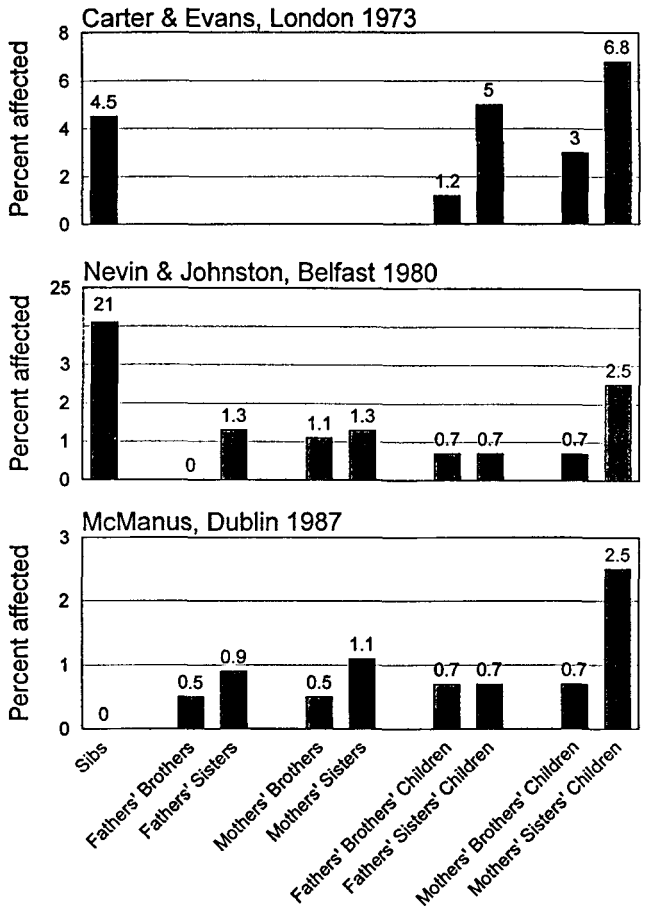

Fig. 1. Risks of neural tube defects in relatives of cases, 3 studies.

In our study we did not completely ascertain first cousins, but only asked if any cousins were affected. However we found no suggestion that mothers' sisters' children have more NTDs. Furthermore, although a trait is transmitted through multiple generations on the mothers' side, that trait is not recurrence risk, but

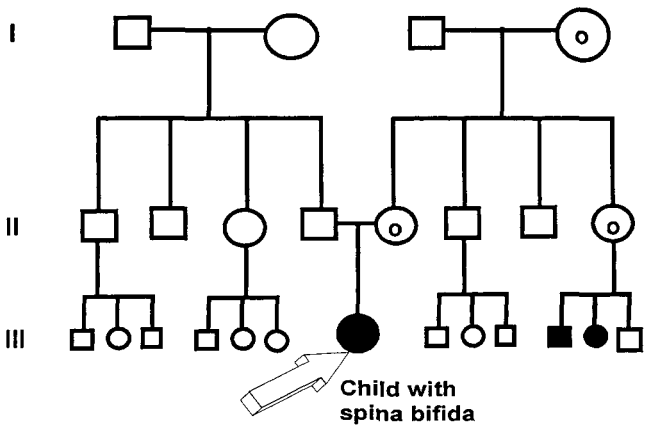

Fig. 2. Hypothetical pedigree with multigenerational maternal inheritance of a trait leading to development of a neural tube defect in the third generation.

related to fertility. Do we interpret this to mean that there are two independent traits segregating in NTD families, one indicated by sibship size, and the other by recurrence risk? It is possible, and it is also possible that the recurrence risk in Italy is too low to detect an association. However, NTDs may have multiple origins with differing risk factors. Studies that include clinical information on the type of lesion may be able to dissect out these patterns [Park et al., 1992]. Studies that found a raised recurrence risk in mothers' sisters' children did not have this information.

Since rates of NTDs vary greatly between geographic locations and recently have declined greatly in formerly high rate areas, it would be unwise to assume that these patterns are unvarying across time and place. In the studies illustrated in Figure 1, for example, there is a 15-year difference in time and difference in geographic locations. Patterns of familial transmission are necessarily confounded with geographic and secular trends.

Recently, in multiply affected families [Chatkupt et al., 1992; Mariman and Hamel, 1992], more maternal gene carriers, or transmitting parents were identified than paternal gene carriers. Information on the level of the lesion was not reported. These authors suggest that maternal imprinting may be involved in the origins of NTDs. Our findings of multigeneration maternal transmission are consistent with genomic imprinting. Another line of investigation that should help in understanding the epidemiologic patterns is the association of the first mutation in a variant of the gene for methylenetetrahydrofolate reductase (MTHFR) with the occurrence of NTDs [Ou et al., 1995; van der Put et al., 1995; Whitehead et al., 1995].

Studies of the genetic origins of neural tube defects have not been common. In a study of miscarriages, Byrne and Warburton [1985] found that there were many more chromosomally normal miscarriages with NTDs in high rate areas and very few in low rate areas. In contrast, the proportion of aneuploid NTDs in miscarriages was about the same regardless of the rate at term. It is possible that the pronounced area differences seen in rates of NTDs among miscarriages might be due to an excess of chromosomally normal miscarriages in high rate areas. Many euploid and aneuploid syndromes carry NTDs as a component. For this reason, we obtained and karyotyped some of the tissue involved in the lesion obtained at surgery. None of the 18 specimens studied showed an abnormal karyotype [Haupt et al., 1995].

Several threads of evidence are converging to paint a picture of a number of different processes causing different types of NTDs. The central event, closure of the neural tube, may occur in a tightly controlled sequence [van Allen et al., 1993] which may vary slightly from males to females [Seller, 1995] and must be controlled by several developmental genes. The PAX family of developmental genes may regulate the developing neural structures [Goulding and Paquette, 1994]. But involvement of PAX-3, the human homologue of the mouse gene believed to play a role in Waardenburg syndrome, one of whose components is spina bifida [Tassabehji et al., 1992; Hol et al., 1995] has been ruled out in multiply affected families [Chatkupt et al., 1995]. Action of the MTHFR variant as the basis for the effect of folic acid will become clearer in the future. The influence of hereditary factors in the genesis of NTDs is subtle and complex and, as we have shown here, involves disturbances in fertility and preferential maternal transmission. Comprehensive studies that include complete family histories with detailed clinical information and molecular studies will be needed to fully understand these relationships.

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